months (range, 1.6 to 31.2 months). There was no correlation between survival and radiotherapy dose, sex, pretreatment WHO performance status and tumor localization.

Our results confirm poor prognosis in glioblastoma multiforme. New more effective therapeutic approaches are sorely needed in this tumour.

ANALYSIS OF MUTATIONS IN TUMOUR SUPRESSOR GENE P53 IN BREAST CANCER PATIENTS FROM POZNAN AREA

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The p53 is transcriptional factor that enhances the rate of transcription of six or seven known genes which play important role in cell cycle regulation. The human p53 protein contains 393 amino acids and has been divided structurally and functionally into four domains. The p53 gene and its protein product have been studied since it became clear that slightly more than 50% of human cancers contain mutations in this gene. A study of mutational spectrum at the p53 gene are localized predominantly in the DNA-binding domain of the protein (exons 4-9). The nature of this changes is most commonly a missense mutation in one allele followed by a reduction to homozygosity, producing a faulty protein. Deletions or chain termination mutations are more rarely.

Mutations in p53 gene have been also found in breast cancer in 30-40% of cases. Kind of these mutations suggest that enviromental mutagens may play important role in arising of this type of cancer.It is observed that in West Poland breast cancer occures more frequently then in other areas of the country; the highest numbers of cases are found in GreatPoland still now for unknown reasons. In this work 48 cases of breast cancer were studied. 12 different mutations in p53 were found. This mutations were then compared with datebase catalogs containing mutations in p53. Only 3 from 12 found mutations are the same as reported in datebase. Nine of them were not observed before what may suggest that specific mutational spectrum in patients with breast cancer from GreatPoland exists. Futher studies involving greater number of cases are needed to confirm this observation.

IS ACUTE MUCOSITIS DOSE LIMITING FOR ALTERED FRACTIONATED RADIOTHERAPY ?

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There is now a substantial number of studies on radiotherapy for head and neck cancer using altered fractionation schedules.

Accumulated dose/week (AD) vs incidence and severity of acute mucositis

In conventional radiotherapy given in 1.6-2.0 Gy fractions up to total dose of about 70 Gy, confluent mucositis (CM) is generally reached at day 22. The threshold for the CM appears to be around 20 Gy and the CM usually develops about 9 days after delivering that dose. However, some studies suggest that the onset of CM may depend on accumulated dose/week and the larger AD is the sooner CM is reached.All these observations suggest that the intensity of acute epithelial reactions, and likely other H-type-like tissues reflects the balance between the rate of cell killing by irradiation and the rate of regeneration of surviving stem cells. Once a critical level of survival cells has been attained, a certain type of clinical damage will develop at a rate only determined by the cellular kinetics of the tissue. When a peak in the CM is reached, further stem cell killing can not produce an increase in intensity of acute reactions, but could be manifest as prolonged time to heal the reactions.

Accelerated and hyperfractionated schedules

Analysis of the sets of data of accelerated, predominantly accelerated and hyperfractionated radiation treatments shows that, except with hyperfractionation and short single course accelerated regimens, the AD is not constant in consecutive weeks of treatment. High AD, above 25 Gy is typical for accelerated treatments when the dose is condensed into a single course in a short overall treatment time.

Conclusions

1. When fractionation regimens are altered to achieve a therapeutic gain through an increased tumour response relative to late normal tissue response, acute mucosal reactions become dose limiting in radiotherapy for head and cancer.
2. Acceptable risk of acute mucositis can be expected when the Dose-Time Ratio (DTR) is lower than 2.5 Gy x day -2 and accumulated dose per week (AD) is less than 12 Gy. Higher AC can only be considered if it is administered in no more than 2 consecutive weeks of 5-6 week treatment or 2-3 week split is given between series of high AD (or DTR).
3. High constant value of the AD (>14 Gy) during 5-6 weeks of treatment or the AD above 20 Gy and DTR above 10 Gy x day-2 lead to high risk of persistent confluent mucositis and consequential late necrosis which may occur within 4-8 months after treatment.

EFFECT OF IRRADIATION ON INTERLEUKIN 6 AND SOLUBLE INTERLEUKIN 6 RECEPTOR MODIFIED MELANOMA GENETIC VACCINE

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We have designed phase I/II human melanoma gene therapy clinical protocol. The aim of the study was to actively immunize HLA-A1 and/or HLA-A2-positive patients with melanoma with an admixture of irradiated autologous tumor cells and allogeneic melanoma cells genetically engineered to secrete IL-6 and sIL-6R in order to elicit or enhance specific and nonspecific anti-melanoma immune responses to autologous tumor cells to eradicate distant melanoma lesions. Irradiation of autologous and allogeneic tumor cells is a key step in preparation of cellular vaccine because of two major reasons, (i) it inhibits cell proliferation which is crucial in the case of autologous cells which may form a tumor; (ii) it increases melanoma vaccine immunogenicity. The aim of the study was to estimate the optimal dose of ionizing radiation which will provide sterilization of both autologous and allogeneic melanoma cells and will ensure cytokine secretion.

Human melanoma cells (Mich-1) were transduced with IL-6 and sIL-6R cDNA using double copy bicistronic retroviral vector. Parental and transduced cells were seeded at in six-well tissue culture plates and were irradiated with 10, 50, 100 and 200 Gy. Secretion of both recombinant proteins into culture was analyzed before and 24, 48, 72, 96 h and 6, 7, 10 and 12 days following irradiation. At the same time adherent cells were enumerated, evaluated for viability and proliferation. At 24, 48, 72 and 96 h postirradiation specific IL-6 and sIL-6R mRNA levels were analyzed.

Irradiation of gene modified cells inhibited their proliferation in the dose dependant manner. Dose of 50 Gy sufficiently affected cell proliferation, however, for safety reasons we decided to use the dose of 100 Gy for vaccine preparation. Irradiation did not inhibit secretion of IL-6 and sIL-6R. In contrary, on a per cell basis it significantly increased their secretion which lasted 12 days postirradiation. Interestingly, we did not observe dose or time dependent differences in specific mRNA cellular levels suggesting that increased secretion of both proteins is regulated not on the transcriptional but rather on the posttranscriptional level. Taking all these facts into account we concluded that irradiation of tumor cells may provide an effective and safe approach for gene-modified vaccine preparation.